TYPE III CRISPR-CAS SYSTEM FOR ROBUST RNA KNOCKDOWN AND IMAGING IN EUKARYOTES

Tech ID: 32820 / UC Case 2022-128-0

PATENT STATUS

Patent Pending

BRIEF DESCRIPTION

Type III CRISPR-Cas systems recognize and degrade RNA molecules using an RNA-guided mechanism that occurs widely in microbes for adaptive immunity against viruses. The inventors have demonstrated that this multi-protein system can be leveraged for programmable RNA knockdown of both nuclear and cytoplasmic transcripts in mammalian cells.

Using single-vector delivery of the S. thermophilus Cas3 complex, RNA knockdown was achieved with high efficiency (90-99%) and minimal off-targets, outperforming existing technologies of shRNA- and Cas13-mediated knockdown. Furthermore, unlike Cas13, Cas3 is devoid of transcriptional activity and thus does not induce non-specific transcriptome-wide degradation and cytotoxicity. Catalytically inactivated Cas3 can also be used for programmable RNA-binding, which the inventors exploit for live-cell RNA imaging. This work demonstrates the feasibility and efficacy of multi-subunit CRISPR-Cas effector complexes as RNA-targeting tools in eukaryotes.

SUGGESTED USES

This invention has the potential to completely supplant RNAi and Cas13-based RNA knockdown technologies.

Suggested uses include:

» research purposes requiring gene perturbation at the transcript (RNA) but not genome (DNA) level.
» efficiently knocking down both cytoplasmic and nuclear RNAs.
» RNA imaging in live cells.
» RNA detection for diagnostic purposes.
» utilizing the enzyme’s ssDNase and/or cyclic oligoadenylate synthesis activities.

ADVANTAGES

The advantages include:

» yields robust, highly efficient RNA knockdown with minimal off-targets and no cell toxicity
» cheap, easy to use, and easy to deliver as an all-in-one vector
» allows for gene perturbation at the transcript (RNA) level
» efficiently knocks down both cytoplasmic and nuclear RNAs

RELATED MATERIALS

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OTHER INFORMATION

KEYWORDS
siRNA, shRNA, knockdown, ncRNA, RNA, Type III, CRISPR, Cas, Csm, Cas13, RNAi

CATEGORIZED AS

» Biotechnology
» Genomics
» Health
» Engineering
» Imaging
» Molecular
» Medical
» Diagnostics
» Gene Therapy
» Imaging
» Research Tools

RELATED CASES

2022-128-0
ADDITIONAL TECHNOLOGIES BY THESE INVENTORS

▶ COMPOSITIONS AND METHODS FOR IDENTIFYING HOST CELL TARGET PROTEINS FOR TREATING RNA VIRUS INFECTIONS
▶ Lentivirus-like Particle Delivery of CRISPR-Cas9 & Guide RNA for Gene Editing
▶ Genome Editing via LNP-Based Delivery of Efficient and Stable CRISPR-Cas Editors
▶ Cas12-mediated DNA Detection Reporter Molecules
▶ Improved guide RNA and Protein Design for CasX-based Gene Editing Platform
▶ Cas13a/C2c2 - A Dual Function Programmable RNA Endonuclease
▶ RNA-directed Cleavage and Modification of DNA using CasY (CRISPR-CasY)
▶ CasX Nickase Designs, Tans Cleavage Designs & Structure
▶ A Dual-RNA Guided CasZ Gene Editing Technology
▶ CRISPR-CAS EFFECTOR POLYPEPTIDES AND METHODS OF USE THEREOF ("Cas-VariPhi")
▶ Modifications To Cas9 For Passive-Delivery Into Cells
▶ A Protein Inhibitor Of Cas9
▶ RNA-directed Cleavage and Modification of DNA using CasX (CRISPR-CasX)
▶ Compositions and Methods for Genome Editing
▶ Split-Cas9 For Regulatable Genome Engineering
▶ NANOPORE MEMBRANE DEVICE AND METHODS OF USE THEREOF
▶ CRISPR CASY COMPOSITIONS AND METHODS OF USE
▶ Single Conjugative Vector for Genome Editing by RNA-guided Transposition
▶ Improved Cas12a Proteins for Accurate and Efficient Genome Editing
▶ CRISPR-CAS EFFECTOR POLYPEPTIDES AND METHODS OF USE THEREOF
▶ Engineered/Variant Hyperactive CRISPR CasPhi Enzymes And Methods Of Use Thereof
▶ Engineering Cas12a Genome Editors with Minimized Trans-Activity
▶ Methods Of Use Of Cas12L/CasLambda In Plants
▶ Type V CRISPR/CAS Effector Proteins for Cleaving ssDNA and Detecting Target DNA
▶ THERMOSTABLE RNA-GUIDED ENDONUCLEASES AND METHODS OF USE THEREOF (GeoCas9)
▶ Structure-Guided Methods Of Cas9-Mediated Genome Engineering
▶ Endonucleases For Rna Detection And Analysis
▶ Efficient Site-Specific Integration Of New Genetic Information Into Human Cells
▶ CRISPR-Cas Effector Polypeptides and Methods of Use Thereof
▶ Class 2 CRISPR/Cas COMPOSITIONS AND METHODS OF USE
▶ Compositions and Methods of Use for Variant Cas4 Endonucleases
▶ Identification Of Sites For Internal Insertions Into Cas9
▶ Small Molecule Assisted Cell Penetrating Cas9 RNP Delivery
▶ Methods and Compositions for Controlling Gene Expression by RNA Processing